Protein
for life

Book of Abstracts

23-26 October 2016 | The Netherlands

Ben Langelaan and Wouter Hendriks

WAGENINGEN
UNIVERSITY & RESEARCH

IPOP Conference Protein for Life

Congress Centre De Reehorst, Ede, The Netherlands

Wageningen University

Editors:
Ben Langelaan
Wouter Hendriks

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Welcome

Welcome

The *Protein for Life* conference addresses the challenge of how we can guarantee nutrition security for both humans and animals against the background of an increasing global protein shortage.

Dietary protein is an essential part of our food intake and crucial for our health and well-being. Over the past decades an enormous progress has been made in our understanding of how dietary compounds affect gene expression (nutrigenomics), and interact with physiological processes in the body. New technologies (e.g. nanotechnology, non-invasive imaging, metabolomics and metabonomics) are furthermore creating unprecedented opportunities for increasing our understanding of how nutrition can optimize health, production, and metabolic capacity. Such knowledge offers ample opportunities for customisation of food products and feeds for specific end-users (both human and animal). These products must provide not only amino acids, but should also fulfil other aspects like sensory satisfaction, health benefits, nutritional support during disease as well as acceptance on a society level.

The current production system for proteins, however, puts enormous pressure on earth's natural resources. It is expected that this situation will worsen due to the world's population growth and its increasing affluence in combination with the effects of climate change and mineral depletion. A more efficient use of existing protein sources and the development of new protein sources for human and animal consumption might be solution pathways to avoid a global protein shortage.

To assess the applicability and functionality of (novel) protein sources, Wageningen University & Research has initiated in 2012 an ambitious research programme called IPOP Customised Nutrition. Within this programme the role of proteins in targeted nutrition for (groups of) humans and animals has been investigated in 3 research areas:

1. Protein sources and processing: the impact of processing on the nutritional value of (novel) protein-containing food and feed products.
2. Protein sources and gastrointestinal tract functioning: kinetics of degradation and bioavailability of proteins in relation to gut health and intestinal immunity.
3. Customised protein nutrition for end-users: interplay between functional properties, sensory performance and consumer acceptance, with a specific focus on the needs of the elderly.

We are very proud of the results that have been achieved in the Customised Nutrition programme and want to connect the most recent insights and findings of this programme with the broad expertise of industry and knowledge institutes during this *Protein for Life* conference. For this we have made an attractive programme that covers all aspects of sustainable and customised protein nutrition, and offers you an excellent opportunity to meet other people interested in the research on a more efficient and effective use of (novel) protein sources.

We wish you all a very inspiring and fruitful conference,

Ben Langelaan, Wageningen Food & Biobased Research
Wouter Hendriks, Wageningen University Dept. Animal Nutrition

Contents

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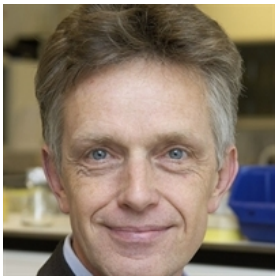

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Communication Services, Events

General Information

Abstracts
These Proceedings contain abstracts of keynote speakers, followed by abstracts of oral presentations, and finally poster abstracts. All posters are arranged in alphabetical order of the first author. The organising committee does not take any responsibility for scientific of typographical errors.

Oral presentations
All presentations will be held in the Congress Centre De Reehorst, Ede.

Posters
All posters are enlisted in alphabetical order in this book. During the whole conference and the poster session they will be on display and can be found in Salon Claire.

Conference Dinner Castle Hoekelum, 24 October, starting 18.30 hrs
Hoekelum estate is an old country house with a charming 18th-century house. It is surrounded by a beautiful pond and special buildings as a separate children's playhouse and a gazebo. The estate is a 10-minute walk from De Reehorst, Bennekomseweg 124, 6721 KE Bennekom.

All participants are invited to join the conference dinner. A great opportunity to network and socialize with other participants while enjoying a delicious dinner.

We gather at 18:00 in the lobby of the Reehorst before departure to the Castle Hoekelum.

Welcome reception, lunches, coffee/tea breaks
Welcome reception will be in Salon Claire.

All coffee/tea breaks and lunches will be served in Salon Claire.

Sponsors
The organizing committee gratefully acknowledges the generous support of the following sponsors:
- Graduate School WIMEK
- Nutricia Research
- Orffa
- Trouw Nutrition
- Nutreco Nederland BV
- Darling Ingredients

Program

Program IPOP Conference Protein for Life

23-26 October 2016

Congress Centre De Reehorst, Ede, The Netherlands

Sunday 23 October 2016

18.00 hrs Pre-registration with drinks

19.00 hrs **Opening lecture**
"Future food and nutrition supply"
Louise Fresco, President of the executive board of Wageningen University & Research

Monday 24 October 2016

08.30 - 09.00 hrs Registration
Wake up coffee/tea

Plenary lecture session (Session sponsored by Darling Ingredients)
Topic: Protein nutrition for humans and animals, global protein supply and requirements
Chairman: prof. Lisette de Groot

09.00 hrs Opening and welcome
Wouter Hendriks, organising committee Protein for Life conference

09.10 hrs **Keynote lecture**
"Alternative protein sources and future protein demand"
Howard Shapiro, Global director of plant science and external research, Mars Incorporated

10.10 hrs "Process development for the production of a novel protein source"
Peter Geerdink, TNO Research, The Netherlands

10.30 hrs **Coffee/tea break**

11.00 hrs "Protein digestion, amino acid bioavailability and nutritional efficacy"
Daniel Tomé, Wageningen University & Research, The Netherlands

11.30 hrs "Eating insects: the challenges of introducing a novel protein source"
Markus Stieger, Wageningen University & Research, The Netherlands

11.50 hrs "Pulse proteins: a sustainable source of proteins"
Fred van de Velde, NIZO Food Research, The Netherlands

12.10 hrs "Water lentils (duckweed) as new plant protein source for human consumption"
Ingrid van der Meer, Wageningen University & Research, The Netherlands

12.30 – 13.30 hrs **Lunch**

Plenary lecture session (Session sponsored by ORFFA)
Topic: The science of proteins (1)
Chairman: dr. Maarten Schutyser

13.30 hrs **Keynote lecture**
"Advances in a description of dietary protein quality for humans"
Paul Moughan, Riddet Institute, Massey University, New Zealand

14.30 hrs "Towards mechanistic understanding of gastric digestion of structured proteins"
Anja Janssen, Wageningen University & Research, The Netherlands

14.50 hrs "Comparison of different methods to assess the nutritional quality of protein"
Sophie van Vliet, Aarhus University, Denmark

15.10 hrs **Coffee/tea break**

Plenary lecture session
Topic: The science of proteins (2)
Chairman: dr. Fred van de Velde

15.40 hrs **Keynote lecture**
"The effect of processing on the rate of protein digestion"
Alan Mackie, University of Leeds, United Kingdom

16.40 hrs "Processing of rapeseed meal: effects on protein hydrolysis and digestibility"
Sergio Salazar-Villanea, Wageningen University & Research, The Netherlands

17.00 hrs "Processing influence on protein digestion and post-absorptive amino acid utilisation in growing pigs"
Tetske Hulshof, Wageningen University & Research, The Netherlands

17.20 hrs "Electrostatic separation of soybean for protein concentration"
Maarten Schutyser, Wageningen University & Research, The Netherlands

17.40 hrs End of scientific part of day 1

18.30 hrs Welcome drinks and start of official conference dinner at Castle Hoekelum

Tuesday 25 October 2016

08.30 hrs Wake up coffee/tea

Plenary lecture session
Topic: Protein and human/animal health
Chairman: prof. Jaap Keijer

09.00 hrs **Keynote lecture**
"Diet and protein in relation to metabolic health"
Susanne Klaus, German Institute of Human Nutrition, Germany

10.00 hrs "Associations between dietary factors and markers of NAFLD in a general Dutch adult population"
Annemarie Rietman, Wageningen University & Research, The Netherlands

10.20 hrs "True ileal amino acid digestibility of a lamb meat hydrolysate and its postprandial metabolic utilization in elderly subjects"
Marco Mensink, Wageningen University & Research, The Netherlands

10.40 hrs **Coffee/tea break**

11.10 hrs "Amino acid requirements of pigs under sub-optimal conditions"
Walter Gerrits, Wageningen University & Research, The Netherlands

11.40 hrs "Use of multi omics approaches in the search for alternative dietary protein sources"
Soumya Kar, Wageningen University & Research, The Netherlands

12.00 hrs "The influence of low protein diets on growth performance and carcass characteristics of gilts or entire boars fed ad libitum"
Meike Bouwhuis, Schothorst Feed Research, The Netherlands

12.20 – 13.30 hrs **Lunch**

Two breakout sessions focussed on the protein nutrition in practice

	1. Learnings from human and animal studies (Session sponsored by Nutricia Research)	***2. Side-streams and insects (Session sponsored by Darling Ingredients)***
13.30 hrs	Chairman: prof. Wouter Hendriks	Chairman: dr. Peter Geerdink
	"Digestibilities of green biomass fractions in monogastrics" Lene Stodkilde, Aarhus University, Denmark	"Animal by-products the unwanted step brother of vegetable by-product streams" Oliver Schneider, SARIA Bio-Industries, Germany

13.50 hrs	"Mitochondrial ATP production and intestinal epithelial permeability – an in vitro model" Lonneke JanssenDuijghuijsen, Wageningen University & Research, The Netherlands	"Insects: a multifunctional product" Maaike Nieuwland, TNO Research, The Netherlands
14.10 hrs	"Protein digestion kinetics of different protein sources in pigs" Hsuan Chen Wageningen University & Research, The Netherlands	"Temperature as decisive factor for protein feather meal" Xinhua Goerner-Hu, Wageningen University & Research, The Netherlands
14.30 hrs	"Effects of a 12-week intervention with protein-enriched foods and drinks on protein intake and physical performance of older patients during the first 6 months after hospital release" Janne Beelen, Wageningen University & Research, The Netherlands	"Lowering protease inhibitors in spray dried eggs improves performance of weaned piglets" Elian Verscheijden, Denkavit Nederland BV, The Netherlands
14.50 hrs	"In vivo digestibility of feather hydrolysates in trout" Claire Butre, Wageningen University & Research, The Netherlands	"Effect of gel structure on the in vitro gastric digestion of plant proteins" Mauricio Opazo-Navarrete, Wageningen University & Research, The Netherlands
15.10 hrs	**Coffee/tea break**	
15.40 hrs	"Applying mealtime functionality for tailoring protein enriched meals to older consumer segments" Louise den Uijl, Wageningen University & Research, The Netherlands	"In vivo and in vitro study on the mode of action of spray dried plasma when used as feed additive" Marcel Hulst, Wageningen University & Research, The Netherlands
16.10 hrs	"Novel protein foods: what do elderly need, what do elderly want?" Lotte van der Zanden, Wageningen University & Research, The Netherlands	"Sustainable protein production using lesser mealworms" Natasja Gianotten, Proti-Farm R&D BV, The Netherlands
16.30 hrs	Poster session and networking with drinks and bites	

Wednesday 26 October 2016

08.30 hrs Wake up coffee/tea

Plenary lecture session
Topic: Future challenges for sustainable protein supply and consumption
Chairman: prof. Harry Wichers

09.00 hrs **Key note lecture**
"Sustainability and Protein Provision: Drawing on a Dozen Different Disciplines
Harry Aiking, Vrije Universiteit Amsterdam, The Netherlands

09.45 hrs "Exploring dietary guidelines reflecting both climate impact and nutritional values"
Corné van Dooren, Voedingscentrum, The Netherlands

10.15 hrs "The effect of exercise on intestinal permeability towards small molecules and protein"
Lonneke JanssenDuijghuijsen, Wageningen University & Research, The Netherlands

10.35 hrs **Coffee/tea break**

11.00 hrs "Sustainable protein supply for animal nutrition"
Wouter Hendriks, Wageningen University & Research, The Netherlands

11.30 hrs "Creating synergy: the industry outlook"
Gerard Klein Essink, Bridge2Food, The Netherlands

12.00 hrs Discussion and closing remarks
Ben Langelaan, Organizing Committee Protein for Life Conference

12.30 hrs Lunch and departure of conference delegates

13.30 hrs End of conference

Poster presentations

"Impact of free lysine on reactive lysine measurement by guanidination"
Tetske Hulshof

"Amino acid demand in piglets during post-weaning diarrhoea"
Jan-Willem Resink, Trouw Nutrition BV, The Netherlands

"A hybrid dry and aqueous fractionation method to obtain protein-rich fractions from quinoa"
Geraldine Ruiz

"Intrinsic ^{15}N labelling of bovine milk protein for the development of a dual stable isotope-based method to measure dietary protein quality"
Nikki van der Wielen

Oral presentations

Opening lecture

Sunday, 23 October 2016

OPENING LECTURE

FUTURE FOOD AND NUTRITION SUPPLY

Louise Fresco

President of the executive board of Wageningen University & Research

Since July 2014 Professor Louise O. Fresco is President of Wageningen University and Research, in The Netherlands. She combines a long academic career as professor in Wageningen and Amsterdam, with various visiting professorships, with an extensive involvement in policy and development. She is a member of the Dutch Royal Academy of Sciences and of four foreign Academies, as well as Distinguished Visiting Scholar at the Academy of Sciences of South Africa. She served for nearly ten years as Assistant-Director General at the a Food and Agriculture Organisation of the UN, spent extensive periods in Africa, Asia and Latin America. She is also a member of the Trilateral Commission. In 2014 the EU Commission asked her to chair the Evaluation of the Seventh Framework Program for Research. She received two national prizes - Comenius and Groeneveld - for her work.

Professor Fresco serves as a non-executive director of Unilever, was a member of the supervisory Board of Rabobank and of the Socio-Economic Council, the highest advisory body in the Netherlands. She is a member of the Council of Advisors of the World Food Prize. She is also involved in many philanthropic and cultural foundations, serves as a board member of the Concertgebouw Orchestra, previously a member of the Erasmus Prize Foundation and member of the editorial board of the Dutch literary magazine De Gids.

She has twelve non-scientific books published in Dutch, including three novels. She has a fortnightly column in the leading daily NRC. In 2013, the six-part documentary *Frescos Paradise* was broadcasted on Dutch TV. The series is based on her book *Hamburgers in Paradise. Food in Times of Scarcity and Abundance.* In 2013, Louise O. Fresco published a thought provoking op-Ed in Science magazine *The GMO Stalemate in Europe*. In 2009 she gave a TED talk in Palm Springs and participated in the Nobel Prize Dialogues in Stockholm and Tokyo.

Session 1

Protein nutrition for humans and animals, global protein supply and requirements

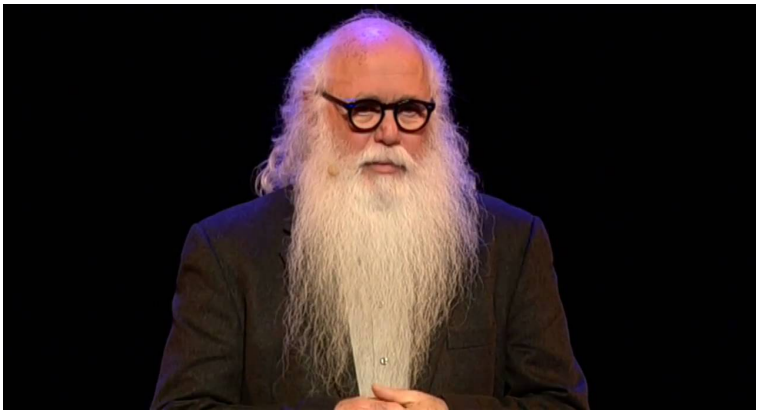
Monday, 24 October 2016

KEYNOTE LECTURE

1.1 ALTERNATIVE PROTEIN SOURCES AND FUTURE PROTEIN DEMAND

Howard Shapiro

Global director of plant science and external research, Mars Incorporated



Within Mars, Incorporated, Dr Shapiro is responsible for the plant science of their primary agricultural products, investigation of potential new plant based solutions for use in brands, review and oversight of existing and future plant based research, co-chair of the Plant Science Pod of the Mars Sustainability Advisory Council, member of the Technical Committee, and leader of the sustainability/production models for agroecological, agroforestry and agroeconomics of multifunctional cacao systems globally. He is Adjunct Professor in the College of Agriculture and Environmental Sciences, The University of California, Davis.

He has twice been a university professor, twice a Fulbright Scholar, twice a Ford Foundation Fellow and winner of the prestigious National Endowment for the Humanities Award. He has worked with indigenous communities, non-governmental organisations, governmental agencies and private institutions throughout the world and many national and regional agricultural institutions as an advisor and policy maker including, but not limited to, ACDI-VOCA, Winrock International, Gates Foundation, AFD, World Bank, UNDP-GEF, United States Department of Agriculture - Agricultural Research Service, United States Agency for International Development, United States Forest Service, ICRAF (The World Agroforestry Centre), Conservation International, WWF, International Institute for Tropical Agriculture, The International Maize and Wheat Improvement Center (CIMMYT) and more.

Prof Shapiro is the author of three books and he is currently co-authoring three books, Chocolate: History, Culture and Heritage (published date 16 February 2009); the Science of Theobroma cacao: Botany, Chemistry & Medicine (publication date spring 2010); and the Future of Agroforestry and Landuse Globally (publication date fall 2010).

1.2 PROCESS DEVELOPMENT FOR THE PRODUCTION OF A NOVEL PROTEIN SOURCE

Geerdink, P.[1] (peter.geerdink@tno.nl), Sebastian, P.J.[2], De Jong, G.A.H.[1], Bussmann, P.J.TH.[1]

[1] Institute for applied scientific research (TNO), The Netherlands
[2] Wageningen University and Research Centre (WUR), The Netherlands

Background
The world population will grow towards 2050 and onwards. One of the great challenges coming with this growth in population is the production of nutritious food in sufficient quantities. The most important factor in this food is protein. There is a vast source of protein available in the western world that is up to now hardly utilized.

The protein
The protein from leaves, mainly the enzyme RuBisCo, is nowadays not used as a food ingredient, although its nutritional and functional properties show great promise to be integrated in the food chain. In order to demonstrate the potential of this sustainable protein source, TNO has worked towards a protein production process and realized the production of a leaf protein isolate that has a good nutritional value, is highly soluble and functional and possesses no known allergenicity.

The process
This process was developed for sugar beet leaves, but has now been adapted to process a spinach side stream from vegetable processing facilities. Furthermore the process has been optimized to produce a very white and pure protein by addition of pectinase and PVPP. These adaptations in the processing route also improve the performance of some of the unit operations, like the centrifuges and filtration equipment. From this juice fraction a white, soluble, functional and highly purified dry protein is produced.

The product
The gelling capacity is the most promising property of the protein, being more than four times better than whey protein and ovalbumin. This will allow applications in for example meat replacers, in which both gelling and nutritional value are of key importance.

Outlook
At the end of 2016 the preparations will be made for the construction of a continuous protein production facility in France. This facility will produce protein from approximately 4.000 kg of spinach side stream per hour.

1.3 PROTEIN DIGESTION, AMINO ACID BIOAVAILABILITY AND NUTRITIONAL EFFICACY

Tomé, Daniel

AgroParisTech, INRA, Paris, France, Wageningen University & Research, The Netherlands

Dietary protein supplies the body with nitrogen and amino acids. Amino acids are used to synthesize and maintain around 10 kg body protein as well as other non-protein nitrogenous substances. Among the 20 amino acids that constitute the proteins, 9 are considered as indispensable and must be provided in the diet. Current discussion of International Authorities (WHO, FAO, IOM, EFSA) are related the Protein Digestibility-Corrected Amino Acid Score (PD-CAAS, FAO/WHO/UNU 2007 as the reference score for assessing protein quality. In the PD-CAAS indispensable amino acid content is corrected by protein digestibility and related to a reference amino acid profiles considered to meet indispensable amino acids needs. The digestibility of protein has largely been determined from faecal digestibility but As unabsorbed amino acids are mostly metabolized by colonic bacteria and converted to ammonia and other compounds that can be absorbed, faecal digestibility can be over-estimated, particularly for low digestibility proteins. The ileal digestibility is considered more accurate for dietary amino acid digestibility and availability. In addition, in the PD-CAAS approach the same digestibility of the protein is applied to each amino acid. As all amino acids are not similarly absorbed in a same dietary protein source, a tentative modified AA scoring approach remains discussed (Digestible Indispensable Amino Acid Score – DIAAS, FAO, 2011, 2014) in which the specific ileal digestibility of each amino acid is considered, but it is not used due to methodologic issues. Lastly, the nutritional efficacy of a protein meal can be directly related to dietary amino acids nitrogen retention in the body (NPU - Net Protein Utilization). This retention depends on indispensable amino acid supply but also on the kinetic amino acid absorption that influences the disposal and metabolic utilization of amino acids. It is influenced by the solubility of the protein and by the food matrix. The form of delivery of dietary amino acids constituted an independent factor of modulation of their postprandial regional metabolism with a fast supply favoring the splanchnic dietary nitrogen uptake over its peripheral anabolic use.

1.4 EATING INSECTS: THE CHALLENGES OF INTRODUCING A NOVEL PROTEIN SOURCE

Markus, Tan, Hui Shan, Grace[1]; Fischer, Arnout R.H.[1];
Van Trijp, Hans C.M.[1]; Stieger, Markus (markus.stieger@wur.nl)[1]

[1] Wageningen University, The Netherlands

Interest in the use of insects as human food lies in its excellent nutritional and sensory value, and its environmental advantages over traditional livestock production. While numerous insect species are valued as traditional delicacies in cultures where insects are eaten, on the contrary, most Western consumers reject the idea of eating insects despite being increasingly informed of the potential benefits. This interdisciplinary research explores the cultural and psychological factors underlying consumer attitudes towards eating insects, and examines the sensory perceptions of insect-based foods. First, 8 cross-cultural focus groups were conducted in Thailand and The Netherlands to explore the differences in motivations, perceptions and behavior of consumers and non-consumers in insects. Findings revealed that cultural exposure and individual experiences determined what foods are perceived to be appropriate for consumption and how they should be appropriately prepared. Second, a taste experiment (n=203) with beef burgers labeled to contain unusual novel ingredients including insects demonstrated that the negative taste expectations have little impact on the actual perceived taste, but the perceived inappropriateness for consumption contributes to a low willingness to eat the burgers again. Third, a survey-based study (n=976) and taste experiment (n=135) investigating the role of the product preparation revealed that choosing a tasty and appropriate method of preparation for insects that tastes good in combination is important if consumers are to consider eating it again. Overall, it was observed across studies that Western consumers may be willing to try insect-based foods out of curiosity or due to environmental motivations, but the adoption of insects as food depend very much on other practical and socio-cultural factors beyond the product and sensory factors. This research highlights the numerous challenges that need to be overcome in order for this novel protein source to be introduced in the West.

1.5 PULSE PROTEINS: A SUSTAINABLE SOURCE OF PROTEINS

Pouvreau, Laurice[1] (LAURICE.POUVREAU@NIZO.COM), Kosters, Hans[1], Van de Velde, Fred[1,2]

[1] NIZO food research, the Netherlands
[2] HAS University of Applied Sciences, the Netherlands

Understanding plant proteins is crucial to designing the proper extraction and drying conditions. An ongoing inventory of commercial plant protein ingredients showed that very often plant proteins exhibit a low solubility. Within NIZO's laboratory, studies have been undertaken on protein extraction from green peas (*Pisum sativa*). The protein extracts were heat treated under different conditions and dried by freeze drying and spray drying under different conditions (heat loads). Pea proteins were chosen based on two aspects: (1) Their good nutritional values (balanced amino acid composition, with high level of lysine residues when comparing to other plant proteins) and; (2) Being lactose and gluten free as well as being non-GMO. However, peas -the only legume used for the commercial production of protein isolates -does not have the highest protein yield per hectare. Both lupin and broad beans generate higher yields. The natural symbiosis of legumes with nitrogen-fixating bacteria makes them suitable for inclusion in a crop rotation system. Therefore, these crops are promising, sustainable sources of dietary protein.

This presentation will show the results of the protein extraction of high functional protein from different legumes that can be grown on open fields in northwestern Europe. The various starch-containing crops show similar behavior during processing. Just like peas, the other leguminous crops can be used for the production of both protein and starch. Moreover, the impact of heat treatment and drying conditions on the functionality of pea protein will be shown. Differential scanning calorimetry (DSC) was used to determine the denaturation enthalpy of the different powders, giving an indication for the degree of the "native" state of the protein. As hydrolysis is an proven strategy to improve the functionality and/or applicability of proteins in generally, preliminary results on the hydrolysis of pulse proteins will be presented.

1.6 WATER LENTILS (DUCKWEED) AS NEW PLANT PROTEIN SOURCE FOR HUMAN CONSUMPTION

Van der Meer, Ingrid[1] (INGRID.VANDERMEER@WUR.NL); Van der Werf, Adrie[1]; Mes, Jurriaan[2]

[1] Wageningen University & Research, Plant Research, The Netherlands
[2] Wageningen University & Research, Food & Biobased Research, The Netherlands

Knowing that the world will encounter a problem to provide all people with enough proteins in due time, the global trend is to study new, sustainable protein sources. Our research focusses on the development of land-based aquatic farming systems using duckweed, or water lentils, for human consumption. Duckweed can produce up to ten times more protein per hectare compared to soybean, and is seen as one of the most promising new multipurpose protein crops. It is one of the fastest growing plants on earth and has a high protein content with a good amino acid profile, comparable to soybean. Besides that, it has no agricultural land uses and can be cultivated with no or low chemical input. However, at this moment there is no legal authorization of Duckweed for food applications falling under the Novel Food regulation. Many issues on the nutritional value or potential anti-nutritional value, digestibility, human tolerance, health aspects and consumer acceptance of duckweed as protein source for human consumption still need to be analysed.

Our research goal is: 1) to select a productive and protein rich duckweed ecotype and set up a small scaled hygienic production system; 2) perform biochemical analysis to study risk and health compounds (e.g. Ca-oxalate or heavy metals which might accumulate when grown under unfavourable conditions), potential health supporting compounds (e.g. vitamins, carotenoids) and proteins (e.g. homologs of allergens or anti-nutritional lectins, SOD); 3) study the tolerance of humans for single dose exposure and 4) study the digestibility and nutritional value of the protein fraction by *in vitro* and*in vivo* analysis, the last based on human plasma amino acids levels after a single exposure. These data can be used to support a Novel Food dossier for duckweed and will hopefully show the enormous potential of this crop for human nutrition.

Session 2

The science of proteins (1)

Monday, 24 October 2016

KEYNOTE LECTURE

2.1 ADVANCES IN A DESCRIPTION OF DIETARY PROTEIN QUALITY FOR HUMANS

Paul Moughan

Riddet Institute, Massey University, New Zealand

Prof. dr. Moughan graduated PhD from Massey University in the area of mammalian protein metabolism in 1984. His early research career focused on digestive physiology and the mathematical modelling of amino acid digestion and metabolism in monogastric species of animal, especially avian, porcine, feline and human. Over the last 20 years he has led a systematic discovery-based research programme into the effects of diet on gut metabolism and digestion and, amongst other discoveries, is credited with establishing the role of food peptides in influencing gut protein metabolism. He has also made significant contributions to knowledge in the chemical analysis of foods and the development of bioassays of nutrient availability. The latter have had considerable application in practice. He is widely regarded as a world authority on mammalian protein metabolism and food evaluation science. He was a member of the 2001 FAO/WHO/UNU Expert Consultation to review the protein and energy requirements of humans and in 2011 he chaired the FAO Expert Consultation to review recommendations on the characterisation of dietary protein quality for humans. He is a Fellow of the Royal Society of New Zealand and the Royal Society of Chemistry, Cambridge, England and has published over 400 works of scholarship. In 2012 he was awarded the New Zealand Prime Minister's Science Award and in 2014 an Honorary Doctorate from the University of Guelph.

The world's population is growing rapidly such that by 2050 it is estimated that the world will need to produce 70% more food than it does today. Much of the growth in population numbers will occur in developing nations and it is also expected that with accompanying economic growth in these countries there will be a burgeoning middle class. As the middle class expands there is an increased relative demand for high protein foods such as eggs, fish, meat and

dairy. This trend augments other trends that point to an escalating future demand for food proteins. Already, however, close to 800 million humans suffer from protein/energy malnutrition, so the challenge to adequately feed the world's population will be formidable. The ability to use all food nutrients, but especially protein, wisely will become critical. The presentation will review recent sweeping changes that are being implemented to allow a new and more accurate description of food protein quality. A recent FAO Expert Consultation on the subject of Dietary Protein Quality for Humans has led to three significant changes to the recommendations as to how the quality of food proteins should be described quantitatively. Firstly, it is recommended that in dietary protein quality evaluation, dietary amino acids should be treated as individual nutrients and wherever possible data for digestible amino acids should be given in food tables on an individual basis. This may seem on the surface to be a straightforward recommendation, but may have quite profound implications in practice. Secondly, new recommendations have been made for determining amino acid digestibility and lysine availability in foods. Digestibility is to be determined based on the true ileal digestibility of each amino acid singly and for foods susceptible to protein damage during processing, lysine availability is to be determined based on the true ileal digestibility of reactive lysine. The growing pig replaces the laboratory rat as the preferred animal model for humans in determining protein and amino acid digestibility. Thirdly, a new protein quality score called digestible indispensable amino acid score (DIAAS) is to replace the previous PDCAAS, and for regulatory purposes two scoring patterns have been recommended. The implications of these new recommendations for the assessment of dietary protein quality will be discussed.

2.2 TOWARDS MECHANISTIC UNDERSTANDING OF GASTRIC DIGESTION OF STRUCTURED PROTEINS

Janssen, Anja E.M. (anja.janssen@wur.nl), Luo, Qi, Boom, Remko M.

Wageningen University and Research Centre, Food Process Engineering, The Netherlands

Proteins are essential macronutrients and the digestion starts in the stomach. Numerous*in vitro* studies on protein digestion are based on experiments with dissolved proteins. However, the majority of protein exists in solid food. Therefore, our research is aimed at understanding how the structure of food affects the digestion of protein. We previously studied the *in vitro* digestion of protein and protein gels by analyzing the peptide distribution after hydrolysis, and found that the kinetics of protein hydrolysis in solution and in gels is different. While the dissolved proteins were hydrolyzed through a 'zipper' type mechanism, the gels followed a slower 'one-by-one' mechanism. We hypothesized that pepsin needs to penetrate the gel microstructure and hydrolyze proteins in gel matrices. Thus the digestion kinetics may be limited by diffusion of pepsin in gel matrices, which can explain the differences in hydrolysis kinetics.

We are currently working on better understanding of the gastric digestion of protein gels. Fluorescence Correlation Spectroscopy (FCS) was applied to investigate the diffusivity of pepsin in gel matrices. Scanning Electron Microscopy (SEM) was used to study the surface of undigested and digested gel. We aim at quantifying the microstructural changes of various protein gels during the digestion process.

Knowledge on the kinetics of the pepsin hydrolysis and modelling of the system, combined with techniques such as SEM and FCS should give us insight in the underlying mechanisms of structured protein digestion. By quantifying the diffusion of pepsin, we gained more insight on the action of pepsin and effect of gel structure in protein digestion. Moreover, this approach makes it possible to bridge the digestion process with established physical-chemistry theories and models, which may lead to better knowledge on the underlying mechanisms of gastric digestion.

2.3 COMPARISON OF DIFFERENT METHODS TO ASSESS THE NUTRITIONAL QUALITY OF PROTEIN

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Over the years, nutritional quality of protein has been assessed by different methods, and for many years the protein digestibility-corrected amino acid score (PDCAAS) has been the preferred method to evaluate protein quality in human nutrition. This method uses faecal protein digestibility determined in a rat bioassay, to correct the amino acid content in a test protein. Recently, an expert committee appointed by FAO has proposed to replace PDCAAS with the digestible indispensable amino acid score (DIAAS), in which the true ileal digestibility of every single amino acid is used to correct the amino acid content. DIAAS values above 100% are not truncated in contrast to PDCAAS values, thereby making it possible to evaluate the ability of a protein source to balance the wanted amino acid profile in a mixture with an inferior protein source.

This study consisted of three experiments determining DIAAS and PDCAAS values for milk protein and protein in soya flour, using the amino acid scoring pattern of children between 0.5-3 years of age as a reference. Ileal digestibility was determined in intact and cannulated pigs, respectively, and faecal digestibility was determined in a rat bioassay.

DIAAS for milk protein were 1.28 and 1.29 determined in intact or cannulated pigs, respectively, the untruncated PDCAAS value determined in rats was slightly higher (1.32). For soya flour the DIAAS were 0.93 and 0.94, respectively, and the PDCAAS 0.96. The first limiting amino acid differed between the pig and rat experiments. In milk protein threonine and sulphuric amino acids were the first limiting amino acids, respectively, and in soya protein sulphuric amino acids and lysine.

In this study digestibility estimates obtained in intact or cannulated pigs did not differ. However, standard errors of means were substantially lower for estimates from cannulated pigs.

Plenary lecture session
The science of proteins (2)

KEYNOTE LECTURE

2.4 THE EFFECT OF PROCESSING ON THE RATE OF PROTEIN DIGESTION

Alan Mackie

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Alan Mackie, University of Leeds, United Kingdom, joined the Institute of Food Research in 1983. Since then he has worked on Interfacial layer composition, where the work was key to understanding how caseinate emulsions (common food systems) are stabilised and how that stability can be manipulated to give specific textures. This was subsequently focused on protein surfactant interactions: In particular he was interested in understanding how the interaction between proteins and surfactant at interfaces affected colloidal stability to flocculation and coalescence. They showed that proteins and surfactants stabilise colloidal structures in different ways and that these two mechanisms are incompatible and lead to phase separation at the interface known as Orogenic displacement.

More recently he has worked on Colloidal behaviour in the GI tract where his team has shown the ability of dietary fibre to limit hydrolysis products from in vitro digested emulsions from diffusing into intestinal mucus. In this work they have used multiple particle-tracking of latex beads to reveal the microstructure of intestinal mucus. They also show for the first time that the high negative charge imparted be bile adsorption to the oil droplets allows them to penetrate the mucus layer. Recently, he has used MRI to look at the link between gastric behaviour and physiological responses. In particular they show the importance of understanding the effects of the food matrix on rates of nutrient release.

In September 2016 he moved to University of Leeds to continue these studies as chair of colloid chemistry in the School of Food Science and Nutrition. He has over 180 peer reviewed publications, Total citations (non-self) = 4616, Average citations per item = 28.3, H-index = 44.

The nutritional value of proteins have long been recognised but the rise in food related health problems in the western world has led many researchers to look for strategies to decrease rates of nutrient absorption from foods that are still highly palatable.

One approach is to use increased gastric retention times. This can be achieved through differences in the viscosity of gastric contents or by targeted early delivery of nutrients, especially fat to the duodenum where an endocrine response is elicited. Data from a specific human study will be shown in which different processing and structuring of the same macronutrients into either a liquid or semisolid form were used to change gastric emptying rates (Mackie et al., 2013). This was also shown to have an effect on appetite regulation. A second approach is to alter the structural properties of the food in a way that alters rates of hydrolysis more directly. Examples will be shown in which thermal and enzymatic processing of dairy proteins was used to decrease rates of protein hydrolysis both in vivo (Juvonen et al., 2015) and in vitro (Macierzanka et al., 2012). The thermal processing was carried out as a function of varying pH and resulted in gels with varying core particle size. The enzymatic crosslinking used transglutaminase to decrease protease access to the substrate.

These approaches show that specific processing of protein based foods has the potential to control rates of nutrient release and thus improving the health status of consumers.

JUVONEN, K. R., MACIERZANKA, A., LILLE, M. E., LAAKSONEN, D. E., MYKKÄNEN, H. M., NISKANEN, L. K., PIHLAJAMÄKI, J., MÄKELÄ, K., MILLS, E. N. C., MACKIE, A. R., MALCOLM, P., HERZIG, K.-H., POUTANEN, K. S. & KARHUNEN, L. J. 2015. Cross-linking of sodium caseinate structured emulsion with 1 transglutaminase alters the postprandial metabolism and appetite responses in healthy young individuals. *British Journal of Nutrition,* 114**,** 418-29.

MACIERZANKA, A., BÖTTGER, F., LANSONNEUR, L., GROIZARD, R., JEAN, A.-S., RIGBY, N. M., CROSS, K., WELLNER, N. & MACKIE, A. R. 2012. The effect of gel structure on the kinetics of simulated gastrointestinal digestion of bovine β-lactoglobulin. *Food Chemistry,* 134**,** 2156–2163.

MACKIE, A. R., RAFIEE, H., MALCOLM, P., SALT, L. & VAN AKEN, G. 2013. Specific food structures supress appetite through reduced gastric emptying rate. *American Journal of Physiology - Gut and Liver Physiology,* 304**,** G1038-G1043.

2.5 PROCESSING OF RAPESEED MEAL: EFFECTS ON PROTEIN HYDROLYSIS AND DIGESTIBILITY

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[2] Agrifirm Innovation Centre B.V., Royal Dutch Agrifirm Group, The Netherlands
[3] Laboratory of Food Chemistry, Wageningen University & Research, The Netherlands

Hydrothermal processing is a common practice during the manufacture of protein-rich feed ingredients and feeds. This processing step can induce protein damage, reducing nitrogen and amino acid digestibility/utilization. Protein damage may occur due to physical or chemical changes, both affecting enzymatic accessibility for protein hydrolysis. Increasing the toasting time of rapeseed meal (RSM) from 0 to 120 min decreased protein solubility from 31 to 10% and the reactive lysine to lysine ratio from 0.98 to 0.80. Both these changes were positively correlated to a linear decrease in the rate of protein hydrolysis. The rate of protein hydrolysis was decreased by more than half when toasting time was increased. Protein solubility seems to be a key parameter for understanding the decrease in the rate of protein hydrolysis. The soluble protein fraction separated from the hydrothermally treated RSM was hydrolysed 3-11× faster than the insoluble fraction. In the insoluble fraction, formation of both disulphide bonds and Maillard reaction products was noticed, which explain the decrease in the rate of protein hydrolysis with longer toasting times. Rapeseed meals with different degrees of damage (untoasted or toasted for 60 or 120 min) were mixed into diets and reprocessed (mash, pelleted or extruded). The effects of diet processing on nitrogen digestion along the gastrointestinal tract and apparent amino acid ileal digestibility will be discussed further.

2.6 PROCESSING INFLUENCE ON PROTEIN DIGESTION AND POST-ABSORPTIVE AMINO ACID UTILISATION IN GROWING PIGS

Hulshof, T.G.[1,2] (tetske.hulshof@wur.nl), Van der Poel, A.F.B.[2], Hendriks, W.H.[2], Bikker, P.[1]

[1] Wageningen UR Livestock Research, Wageningen, the Netherlands
[2] Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands

During (over-)processing, reducing sugars can react with the free ε-amino group of lysine to form Maillard reaction products. Processing is known to reduce amino acid (AA) digestibility but it is unknown whether this is caused by a reduction in nitrogen solubility in chyme. Moreover, the post-absorptive effects of processing are unknown. Therefore, a series of experiments was conducted to determine the effects of processing on protein and AA digestion in the small intestine and post-absorptive utilisation of AA for retention in growing pigs. Soybean meal and rapeseed meal were used as protein sources. A model processing method, i.e. toasting at 95°C for 30 minutes in the presence of a sugar-rich lignosulfonate, was used to induce contrasts in protein quality. This was quantified by the reactive lysine content in the protein sources. The first study determined the standardized ileal digestibility (SID) of protein and AA of the protein sources using growing pigs fitted with an ileo-cecal valve cannula. Processing decreased the SID of protein and AA with the largest effect on SID of lysine and reactive lysine. The SID AA contents were used to formulate the diets of the second study. The second study determined the effects of processing on (1) nitrogen solubility in chyme, (2) apparent digestibility of protein along the small intestine, (3) body AA composition, and (4) post-absorptive AA utilisation for retention using a slaughter trial with growing pigs. Processing reduced apparent digestibility of protein especially at the end of the small intestine but this was not explained by effects on nitrogen solubility. Processing reduced AA retention and the lysine content in body protein. Processing reduced the post-absorptive AA utilisation, i.e. use of ingested SID AA for AA retention, for soybean meal but not for rapeseed meal. The interpretation and implications of these results will be addressed.

2.7 ELECTROSTATIC SEPARATION OF SOYBEAN FOR PROTEIN CONCENTRATION

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Laboratory of Food Process Engineering, Wageningen University, The Netherlands

Dry fractionation by milling and electrostatic separation is a novel mild fractionation process for concentration of plant protein. In contrast to conventional wet extraction aiming at manufacturing of protein isolates, dry fractionation uses no water and much lower energy. Although achieved protein purity with this dry process is limited, previous research with pea and lupine showed that dry enriched protein fractions exhibit highly interesting functional behaviour and proteins retain their native state. The aim of this investigation was to use this novel fractionation method to enrich defatted soybean flour in protein. Our in-house developed pilot-scale separator consisting of feeder, aluminium charging device and separation chamber was employed for this purpose. Impact milled, de-fatted soybean flour was separated into positively charged (protein-enriched) and negatively charged (fibre-enriched) fractions. The protein-enriched fraction was collected from the grounded electrode plate in the electrostatic separator. Impact milling at 3000 rpm classifier-wheel-speed provided optimal subsequent electrostatic separation by releasing sufficient detached protein bodies, whilst limiting too excessive agglomeration. Soybean flour with and without hulls was found to result in similar separation efficiency. Increasing the carrier gas velocity was successful in further enhancing the protein purity of the positively charged protein fraction. The formation of agglomerates showed to be the limiting factor for enrichment in our current equipment, which was concluded from the influence of milling conditions, presence of hull particles and nitrogen carrier gas velocity. With optimised process conditions, the protein content of the original soybean flour could be increased from 42 to 58 g protein / 100 g dry matter. Not only the presence of protein is interesting, but also presence of soluble and insoluble fibres makes this fraction promising as functional ingredient in multiple foodapplications.

Session 3

Protein and human/ animal health

Tuesday, 25 October 2016

KEYNOTE LECTURE

3.1 DIET AND PROTEIN IN RELATION TO METABOLIC HEALTH

Susanne Klaus

German Institute of Human Nutrition, Germany

Prof. dr. Susanne Klaus, German Institute of Human Nutrition (DIfE) graduated PhD from University of Marburg (Germany) with a PhD in animal physiology in 1988.

Her research focus and expertise is on:
- energy metabolism, thermogenesis and obesity
- role of macronutrients and intestinal microbiota in energy metabolism and development of metabolic syndrome
- role of adipose tissue and skeletal muscle in energy metabolism
- mouse models for nutrition, energy metabolism, and aging research nutritional intervention studies

Since 1997 she is professor of Physiology of Energy Metabolism at the University of Potsdam, Department of Nutritional Sciences, and group leader at the German Institute of Human Nutrition in Potsdam (DIfE).

Among the energy providing macronutrients protein has a special role because in contrast to carbohydrates and fat there is no specific storage form of protein in our body. An excess of dietary energy intake leads in the long term to obesity and associated metabolic disturbances such as insulin resistance and cardio vascular disease which can reduce lifespan. This is well documented in different model organisms for an excessive fat and carbohydrate intake but the role of dietary protein is less clear. Overall, high-protein diets are discussed very controversially since long-term consumption could be linked to metabolic and clinical problems, such as loss of bone mass and renal dysfunction. However, there is little evidence that a high dietary protein intake is dangerous to healthy individuals. Quite contrary, high protein diets are currently widely used as a dietary approach in the treatment or prevention of obesity and associated disorders. Dietary protein and/or amino acid supplementations are also considered for treatment and prevention of aging related sarcopenia in order to stimulate protein anabolism.

Increased dietary protein leads to activation of the protein kinase mTOR as a cellular amino acid sensor which results in an inhibition of catabolic and induction of anabolic processes including protein synthesis. On the other hand, inhibition of mTOR pathway signalling has been found to promote longevity in different organisms suggesting overall detrimental effects of high protein and amino acid exposure. Human studies in healthy subjects have also produced controversial data. Using feeding studies in mice we could show that increasing the dietary protein content (HP) of a low fat diet led to slightly decreased glucose tolerance without affecting body composition, energy intake and expenditure, and insulin sensitivity. However, in the context of high fat diet induced obesity, increasing the dietary protein content had beneficial metabolic effects. High dietary protein attenuated the development of high fat diet induced obesity and fatty liver through reduction of food intake. This was associated with increased life span in mice.

Differential effects of different protein sources (e.g. animal vs. plant protein, casein vs. whey protein) have been reported suggesting that the amino acid composition plays a role. There is a general agreement that specific amino acids, mainly non-dispensable (i.e. essential) amino acids mediate these effects. Especially branched chain amino acids such as leucine and their metabolites have been shown to address specific molecular mechanisms. Leucine for example specifically induces mTOR regulated pathways. On the other hand, the list of amino acids eliciting specific molecular affects -such as mTOR activation- when supplemented singularly is steadily increasing. We could show in our mice studies that supplementing a high fat diet with either leucine or alanine (a dispensable amino acid) had similar beneficial metabolic effects which were linked to dietary nitrogen intake. This suggests that at least part of the high protein/amino acid effects are mediated by a common signalling linked to nitrogen metabolism. In conclusion, the complex interaction of dietary protein derived amino acids with cellular energy metabolism and metabolic health is far from being fully understood.

3.2 ASSOCIATIONS BETWEEN DIETARY FACTORS AND MARKERS OF NAFLD IN A GENERAL DUTCH ADULT POPULATION

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Division of Human Nutrition, Wageningen University, NL-6703 HD Wageningen, the Netherlands

Non-alcoholic fatty liver disease (NAFLD), the accumulation of triglycerides within hepatocytes, is considered the hepatic manifestation of the metabolic syndrome. Diet is known to affect liver fat accumulation in humans.

To assess the relationship between dietary intake and fatty liver as scored by the validated Fatty Liver Index (FLI), in a large cross-sectional study among a general Dutch adult population.

1,283 men and women aged 20-70y were included. Dietary intake was assessed using a validated semi-quantitative food frequency questionnaire. FLI was derived from BMI, waist circumference, triglycerides and gamma-glutamyltransferase. Associations were adjusted for energy intake, alcohol intake, age, sex, education, smoking and prevalence of hypertension and diabetes.

In this population (mean age 53.6 ± 11.2y; BMI 25.9 ± 4.0 kg/m2; FLI 35.6 ± 27.8), the prevalence of fatty liver as indicated by an FLI>60, was 22.0%. Subjects in the highest FLI-category were more likely to be male, were older and less physically active. Total protein intake and animal protein intake was positively associated with the highest FLI score vs. the lowest (OR 1.26 per 1 en%, 95%CI 1.16-1.37 and OR 1.28, 95%CI 1.19 – 1.38, respectively), whereas for vegetable protein an inverse association was observed (OR 0.80, 95%CI 0.70 – 0.92). A similar positive association with FLI was observed when total and animal proteins were iso-calorically exchanged for carbohydrates and fat. The Dutch Healthy Diet-index was significantly lower in the high FLI group.

Concluding, subjects in the high FLI group consumed more protein, especially from animal origin, less carbohydrates and less dietary fiber. The presence of fatty liver was associated with a higher intake of animal protein and total fat, soft drinks and snacks, and overall less healthy dietary habits. This suggests that dietary intake may affect development of NAFLD and hence dietary changes may prevent the onset.

3.3 TRUE ILEAL AMINO ACID DIGESTIBILITY OF A LAMB MEAT HYDROLYSATE AND ITS POSTPRANDIAL METABOLIC UTILIZATION IN ELDERLY SUBJECTS

Henare, Sharon[1]; Awati, Ajay[1]; Cui, Jack[1]; Rutherfurd, Shane[1]; Mensink, Marco[2] (marco.mensink@wur.nl)*; Deglaire, Amelie[1,3]; Moughan, Paul[1]

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The objective of the study was to determine the protein quality of an ovine (lamb) meat hydrolysate as assessed by the measurement of its true ileal amino acid digestibility and postprandial utilization. True ileal digestibility was determined in six healthy growing pigs consuming a lamb meat hydrolysate-based synthetic diet for two weeks. To determine postprandial utilization, healthy older adult humans ingested a mixed meal that contained uniformly and intrinsically ^{15}N-labelled lamb meat hydrolysate (n=16), or, for comparison, intrinsically ^{15}N-labelled casein (n=9). The postprandial concentration of dietary nitrogen was measured in blood and urine nitrogen pools. Eight hours after ingestion low deamination losses were noticed, with no differences between lamb and casein. The fraction of dietary N irreversibly transferred to body urea and urinary urea and ammonia was 13.5 ± 1.3 % and 13.0 ± 1.3 % for lamb meat hydrolysate and casein respectively. The incorporation of dietary nitrogen into serum protein at 8 hours postprandial reached 7.6 ± 0.7% and 10.7 ± 0.4% of ingested nitrogen for lamb and casein. The mean true ileal amino acid digestibility of the lamb meat hydrolysate across the examined amino acids was 98.4 ± 0.8%. The estimated net postprandial utilization (NPPU) was 84.9 ± 1.4%, and biological value of the lamb meat hydrolysate was 86.3 ± 1.5%. The results confirm that the lamb meat hydrolysate is a high quality source of protein with a high true ileal amino acid digestibility and has a high postprandial utilization. We conclude that the lamb meat hydrolysate could serve as a protein of high nutritional quality for the older adult human.

3.4 AMINO ACID REQUIREMENTS OF PIGS UNDER SUB-OPTIMAL CONDITIONS

Walter Gerrits

Wageningen University & Research, The Netherlands

3.5 USE OF MULTI OMICS APPROACHES IN THE SEARCH FOR ALTERNATIVE DIETARY PROTEIN SOURCES

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Before new protein sources can be safely exploited in human or animal feeding practice, more knowledge is needed on their interaction with the host, especially at the level of the gastrointestinal tract (GIT). Recent advancements in bio-technological platforms are enabling a wide range of high-throughput molecular analyses to be performed on biological samples, providing new insights into the biological system. We investigated the effects of feeding mice during a period of four weeks on semi-synthetic diets containing 30% of one of one of the following protein sources: soybean meal (SBM), casein, delactosed whey powder, spray dried plasma protein, wheat gluten meal, or yellow meal worm. Multi-omics data was obtained on gene expression in ileal tissue, microbiota composition in the ileal digesta, cytokine levels in blood serum, and metabolomics profiles in serum and urine. A cross–platform multi-omics based analysis was adopted to gain biological insight into possible interactions between different phenotypes. Moreover, we complemented the systems biology analysis with conventional standalone-platform specific data analysis to confirm some of the discovered links and to verify the interpretations. These results uncovered associations between phenotypes and showed how dietary effects are interconnected at both local and systemic level. Analysis of the associations pinpoints the key roles of bacterial family S24-7 in ileal digesta, plasma glutathione and TNF-α in response to dietary protein interventions. Further, results of conventional analysis reveal that the SBM-based diet deviated strongly from the other dietary treatments in its capacity to interact with the host homeostatic response and the residing ileal microbiota. We show that the holistic approach of ~omics based "systems biology" aids in generating novel knowledge on the triangular interactions between nutrition-host-microbiota in GIT by uncovering novel phenotypic associations for further experimental investigation.

3.6 THE INFLUENCE OF LOW PROTEIN DIETS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF GILTS OR ENTIRE BOARS FED AD LIBITUM

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Reducing the dietary crude protein content is a well-known option to limit the environmental impact of pig production. However, it is important to keep on updating the references of such practice, thereby incorporating the evolution of farming practice in order to promote the adoption of this strategy. For this purpose, a trial was performed to determine the effect of a 10% reduction in protein levels during the fattening period on the growth performance and carcass characteristics of gilts or entire boars from two sire lines which were slaughtered at 120 kg live weight. All diets had similar levels of digestible amino acids: 9.43, 7.88 and 6.85 g digestible lysine/kg in starter, grower and finisher diets, respectively. Other amino acids were balanced according to the ideal protein pattern. The experiment involved 16 pens containing 12 pigs each, equipped with feeding stations making it possible to record the daily feed intake of individual pigs. Pigs were weighed individually at the start, then every three weeks until slaughter and carcass grades were collected at the slaughterhouse. Faecal consistency was assessed weekly during the trial. Reducing crude protein provision by 10% did not penalize growth performance or carcass grade. In the finishing period, the feed intake of pigs receiving the low protein diet was higher and their carcass yield was reduced. Pigs receiving the highest protein diet tended to show looser faeces. Expected differences between genders and sire lines were observed on feed intake, growth rate and carcass grade. Lysine marginal efficiency for growth did not depend on sire line, but an effect of gender was detected for each period. The current results confirm that reducing protein supply by 10% did not have detrimental effects on the performance of fattening pigs fed ad libitum.

Session 4
Two breakout sessions focussed on the protein nutrition in practice

1. Learnings from human and animal studies
2. Side-streams and insects

LEARNINGS FROM HUMAN AND ANIMAL STUDIES

Chairman: Wouter Hendriks

4.1.1 DIGESTIBILITIES OF GREEN BIOMASS FRACTIONS IN MONOGASTRICS

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Department of Animal Science, Aarhus University, Foulum, Denmark

Green crops like grass and legumes have the potential to become sustainable protein sources potentiating a feed alternative for the increasing livestock sector. By processing the crops into a solid fraction and a liquid juice from which proteins can be precipitated, one bypasses many of the limitations of direct use of the crop for monogastric feed. In this study, the chemical composition of fractions from locally grown red clover, white clover, perennial ryegrass, and lucerne produced after screw-press processing was analysed. Moreover, digestibility of dry matter and protein was determined in rat experiments.

Protein concentrates with higher protein contents (239-388 g/kg DM) and pulps with lower protein contents (110-216 g/kg DM) than the raw material were produced by processing. Lucerne protein concentrate had the highest content of protein and perennial ryegrass pulp the lowest.

The amino acid compositions were very similar in the different plants and fraction where the average Lys, Met+Cys, and Thr contents were found to be 6.0, 2.7, and 4.7 g/16g N respectively.

In lucerne, perennial ryegrass, and white clover there was a significant difference between the dry matter digestibility of fractions whereas for red clover, the unprocessed plant and protein fraction were similar. The highest protein and dry matter digestibilities were found in lucerne protein concentrates (0.85 and 0.77 respectively) and the lowest in lucerne pulp (0.52 and 0.21 respectively).

The study demonstrates potential of using green crops as protein source for monogastrics because of high protein content, proper amino acid composition, and high digestibility but also demonstrates the need for improvements. Therefore, the study provides valuable results and knowledge to the production of alternative and sustainable protein sources for animal feed.

4.1.2 MITOCHONDRIAL ATP PRODUCTION AND INTESTINAL EPITHELIAL PERMEABILITY- AN IN VITRO MODEL

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In vivo studies suggest that maintenance of intestinal barrier function is dependent on mitochondrial ATP production, but final proof is lacking. We aim to provide the evidence using an *in vitro* model. To establish the relation between mitochondrial ATP production and intestinal permeability it is essential that ATP production is dependent on mitochondria, which is not the case in glucose-rich cell culture media. Our first goal is therefore to establish an *in vitro* intestinal permeability model, where cells are dependent on mitochondrial ATP production, as they are *in vivo*.

Human Caco-2 cells were grown for 10 days in cell culture flasks or for 14 days on transwell inserts in either glucose or galactose medium. Mitochondria were stained and visualized with MitoTracker Green. Cellular respiration was measured with OROBOROS respirometry. Western blot analysis was used to determine levels of OXPHOS proteins. Rotenone was used to induce cellular ATP depletion and monolayer permeability was assessed with trans-epithelial electrical resistance measurement (TEER).

Caco-2 cells grown on galactose show a higher density and network of mitochondria, supported by a 60% increase in cellular respiration and significantly increased OXPHOS protein expression compared to glucose-cultured cells. Rotenone treatment results, in galactose-cultured cells only, in a significant reduction in cellular ATP levels after 24 hours, with concomitant reduction of TEER to 50% of its starting value and no observed toxicity.

We successfully developed a Caco-2 epithelial cell model dependent on mitochondrial energy

4.1.3 PROTEIN DIGESTION KINETICS OF DIFFERENT PROTEIN SOURCES IN PIGS

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Ileal digestibility values for protein and amino acids of protein sources do not provide information on the digestion kinetics of protein and amino acids in the gastrointestinal tract (GIT), which could affect their post-absorptive utilization. The objective of the present study was to evaluate protein digestion kinetics of various protein sources in pigs. The apparent digestibility of crude protein (ADCP) and the mean retention time (MRT) in four equal parts of the small intestine (SI) (i.e. 0-25%, 25-50%, 50-75% and 75-100% of SI) were determined in growing pigs fed diets containing soybean mean (SBM), rapeseed meal (RSM), wheat gluten (WG) and plasma protein (PP) as the only protein source. The protein digestion kinetics of the different protein sources were calculated by relating the ADCP up to each part of the SI to the sum of MRT up to that part of the SI. The resulting curve was then described by an exponential model: $D_t = D_{max}(1-e^{-kt})$ where D_t (%) is the percentage of CP digested at time t (min), D_{max} (%) is the potential digestible CP fraction (asymptote), and k is the rate constant. Up to the end of the SI, WG had the highest ADCP, followed by PP and SBM, and RSM had the lowest ADCP (91%, 86%, 74% and 60%, respectively; $P<0.05$). MRT increased as the digesta transited from the proximal to the distal part of the SI. Digestion rate of CP, was highest for PP (3.38×10^{-2} min^{-1}), followed by WG and SBM (1.57×10^{-2} and 1.54×10^{-2} min^{-1}, respectively) and lowest for RSM (1.36×10^{-2} min^{-1}). In conclusion, PP is digested faster compared to WG, SBM and RSM in the small intestine of pigs.

4.1.4 EFFECTS OF A 12-WEEK INTERVENTION WITH PROTEIN-ENRICHED FOODS AND DRINKS ON PROTEIN INTAKE AND PHYSICAL PERFORMANCE OF OLDER PATIENTS DURING THE FIRST 6 MONTHS AFTER HOSPITAL RELEASE: THE RANDOMIZED CONTROLLED CATER WITH CARE® TRIAL

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Objectives: During and after hospitalisation older adults are recommended to consume 1.2-1.5 g of protein per kg body weight per day (g/kg/d) to improve recovery. We studied the effectiveness of protein-enriched foods and drinks in reaching those goals.

Design, setting, participants, intervention: This randomized controlled trial followed 75 patients of ≥65 years (mean age: 76.8 ± 6.9 years) who took part in the hospital phase of this trial in their first 6 months after hospital release. During the 12-week intervention period at home, subjects in the intervention group received protein-enriched foods and drinks, while the control group received regular foods and drinks.

Measurements: Data were collected the day before discharge and 2, 6, 12, and 24 weeks after hospital release. Protein intake, indicators of physical performance, and body weight were measured.

Results: The intervention group had a higher protein intake during the 12-week intervention period compared to the control group ($P<0.01$): 112 ± 34 g/d (1.5 ± 0.6 g/kg/d) versus 78 ± 18 g per day (1.0 ± 0.4 g/kg/d). Energy intake did not differ between groups (2250 ± 531 kcal in intervention group, 2007 ± 493 kcal in controls, $P=0.070$). Physical performance, gait speed, chair rise time, body weight and nutritional status improved at week 12 compared to baseline (time effect $P<0.05$), but were not different between groups. Leg extension strength, hand grip strength, and independence in activities of daily living did not change up to 24 weeks.

Conclusion: Protein-enriched foods and drinks successfully increased protein intake, but did not improve physical recovery in the first 6 months after hospital release.

4.1.5 IN VIVO DIGESTIBILITY OF FEATHER HYDROLYSATES IN TROUT

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Fish grown in aquaculture are a valuable protein source for human consumption. Most cultured fish demand a high protein content in their feed (>35%). Fish meal is an important source of feed but efforts are made in finding alternatives to avoid using fish meal in fish diets. Feathers are a by-product of the poultry industry, readily available, inexpensive and containing proteins to 85-100 %. Feather meals can thus be used as replacer of fish meal in fish feed. Keratin is the main protein constituent of feathers. The keratin molecule contains a high content of disulphide bridge and is insoluble. Feathers need to be hydrolysed to increase the solubility and digestibility. This processing step as well as the source of the feathers are source of variability in the ingredient. In addition the processing can affect the nutritional quality of the ingredient by reducing the content of essential amino acids with the formation of cross linked amino acids (CLAA). To assess if properties of feather hydrolysates can explain the variation in nitrogen apparent digestibility coefficient (ADC) for fish, 11 feather hydrolysates were characterized and used as ingredient for trout diets in an *in-vivo* digestibility trial. Rainbow trout were fed with 12 diets (11 feather containing diets and the basal diet as control) for 4 weeks in duplicate and fecal matter was collected to determine the ADC of the different nutrients. Feed conversion ratio was 0.88 for the test diets on average and significantly higher than for the control diet (0.80). ADC N of the feather hydrolysate is varying between 46.9 and 95.2 %, showing a large variation in the ADC. It became apparent that the ADC N was correlated with the content in the CLAA lanthionine as well as with the buffering capacity of the feather hydrolysates.

4.1.6 APPLYING MEALTIME FUNCTIONALITY FOR TAILORING PROTEIN ENRICHED MEALS TO OLDER CONSUMER SEGMENTS

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The group of older adults is highly heterogeneous and does not always meet its recommended protein intake. We explored mealtime functionality as a basis for tailoring protein enriched (PE) meal concepts to two senior consumer segments: 1) *cosy socialisers,* who eat mainly for cosiness and social interaction, and 2) *physical nutritioners,* who eat mainly for nutrients and physical needs. We hypothesised an increased 'product-cluster fit' when the functional meal associations are congruent to the clusters' functional mealtime expectations. In a home-use test, participants (N=91, mean age 68.1 (y) ± 5.3 (SD), 44 *cosy socialisers*) prepared and consumed three kale mash meal concepts once over three weeks: (1) a basic meal concept (without PE/tailoring), (2) a cosy meal concept (PE/tailored to mealtime expectations of *cosy socialisers)*, and (3) a physical meal concept (PE/tailored to mealtime expectations of *physical nutritioners)*. The participants reported their expectations and experiences with the recipes and dishes (e.g. expected liking; attractiveness recipe; actual liking; taste; smell; satisfaction). Results showed that the cosy meal concept was experienced as 'traditional' (p<0.05), whereas the physical meal concept was perceived as 'healthy' (p<0.05), trendy (p<0.05), and 'energising' (p<0.09). Nonetheless, the cluster*meal concept effect did not reach statistical significance for any of the outcome variables, indicating a similar actual experience of the congruent and incongruent meal concepts. This study highlights for the first time both the potency and challenges of tailoring PE dishes to specific older consumers and underlines that an increased 'product-cluster fit' is not straight forward to achieve.

4.1.7 NOVEL PROTEIN FOODS: WHAT DO ELDERLY NEED, WHAT DO ELDERLY WANT?

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During the last decades, the elderly population has strongly increased relative to younger populations and this trend is continuing. Companies have become increasingly aware that elderly consumers are worthwhile to direct their attention to, and have started to develop food innovations that take into account the changing nutritional needs of ageing consumers. Especially the development and commercialization of protein-enriched foods may benefit elderly consumers. Marketing such products to older adults has proven to be difficult, however, and four main challenges can be identified: 1) distinguishing between consumers based on their age is arbitrary and encourages age-based stereotyping, 2) the elderly consumer population is strongly heterogeneous and requires a market segmentation approach, 3) segmentation is a delicate practice and does not always result in informative subgroups, 4) the way in which consumers make decisions affects the success of interventions and marketing approaches. To tackle these challenges, it is crucial for marketers and researchers to gain a deeper understanding of the elderly consumer population, which is the central idea behind consumer-oriented new product development. This presentation builds on the framework for consumer-oriented new product development, and provides understanding of elderly and insights in marketing protein-enriched foods to elderly consumers based on a series of studies. A focus group study illustrates various difficulties surrounding the commercialization of protein-enriched foods among elderly consumers. A narrative review evaluates various ways in which the elderly population can be segmented in the functional food market and a segmentation study demonstrates the benefits of segmenting elderly consumers. A field study illustrates that taking into account consumer decision-making strategies can provide useful entry points for health interventions and an online survey demonstrates that age-related differences in decision-making are driven by other mechanisms than sheer age. Remaining gaps in the understanding of elderly consumers are discussed.

SIDE-STREAMS AND INSECTS

Chairman: Ben Langelaan

4.2.1 ANIMAL BY-PRODUCTS THE UNWANTED STEP BROTHER OF VEGETABLE BY-PRODUCT STREAMS

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Bio-refinery of by-products has attracted attention during the last years, focusing mainly on plant based and much less on animal by-products (ABP) materials. However, significant volumes of food grade ABP are available, which are ingredients in food, feed, cosmetics and technical products. This review summarizes the potential of ABP for bio-refineries, looking at volumes, and giving examples for existing refineries from basic rendering to sophisticated separation / extraction methods. In Europe 250Mio pigs, 25 Mio bovines and 7 200 Mio poultry were slaughtered in 2014 (EU-28). These result in 7.5Mio MT porcine, 3Mio MT bovine and 3.25 Mio MT poultry ABPs. These ABPs are majorly rendered in a robust process, comprising crushing, drying and pressing. The results are processed animal proteins (PAP) and fats with both ~15% yield, used for petfood, animal nutrition, technical or edible items. This is Addition to low-end valorizations such as fertilizer or biofuels. Refinery approaches in rendering have been aiming for improved separation, increasing protein levels and increasing digestibility, always with focus on PAP and fat. Only for very limited ABP streams extraction methods have been developed. An important example of a drug obtained from ABP by extraction is heparin, based on pig mucosa. One kg of heparin equals the mucosa derived from more than 7200 pigs. As no material is wasted, protein remainders of the separation are used in pig nutrition due to their biological functionality. Another example is the transformation of bones into nutritious hydrolyzed protein isolates. This process was breaking the paradigm of the basic rendering. Combined with these examples the review discusses safety and quality aspects which will lead to a better appreciation of this raw material stream.

4.2.2 INSECTS: A MULTIFUNCTIONAL PRODUCT

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Most 'Western' people heard of it, some tried it, and everyone has an opinion about it: eating insects. While 80% of the world population eats insects and many species in several places are regarded as delicacy, most Westerners see insects as pest and not food.

Still, the many advantages of insects start to be more and more acknowledged worldwide. Although variance between species is enormous, both in specifications and in consumer acceptance, in general it can be stated that insects are a good alternative to meat because of their high protein and mineral content. Rearing this 'mini-livestock' uses less land and water than rearing conventional livestock. Furthermore, insects have a high feed conversion rate and emit less greenhouse gasses.

No wonder that insect initiatives are popping up all over the place. For example initiatives in Africa, where insects are consumed for ages: catched in the wild when in season. To make healthy protein available year round, the Flying Food project (www.flyingfoodproject.com), lead by TNO has started, in which the whole value chain of rearing crickets in Kenya and Uganda is set up.

At the same time, also in Europe many insect initiatives start, of which Scenoprot is one example. Scenoprot is a six-year project running in Finland, which focusus on decreasing dependency on importing protein and increasing the use of sustainable protein sources. Scenoprot uses a diverse approach, where a range of new proteins is considered and judged based both on viability of growth in Finland, nutritional value and safety. One of the possibilities that is investigated is rearing insects. TNO plays a role both in allergenicity studies as well as in a feasibility study on insect rearing in Finland.

The initiatives vary enormously: a diversity needed to make insects – 'from farm to fork' – a success.

4.2.3 TEMPERATURE AS DECISIVE FACTOR FOR PROTEIN FEATHER MEAL

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If feather meals could be used more efficiently as feed ingredient, a major contribution to ease protein deficits in global animal nutrition would be made. Feather meal has an extremely high protein content compared to soy bean meal or others. Therefore it is a valuable feed ingredient complementary to ingredients such as fish meal. The use of feather meal as feed ingredient has still some drawbacks which need to be overcome i.e., a) unbalanced amino acid composition compared to animal's nutritional needs b) about 30% loss of essential amino acids (EAA), in particular Lys and Thr and Cys as conditionally EAA, , and c) low apparent digestibility of EAA (<50% respectively). Especially the later one is caused by the most common processing method: thermo-pressure hydrolysis however a detailed understanding on the relation between processing parameters and feather meal properties is lacking and will be discussed here.

We varied the temperature (106°C-174°C), residence time (10-110 minutes) and moisture content (53%-87%) during thermo-pressure hydrolysis of chicken feathers. It turned out that temperature has the most significant influence on the properties of the produced meal. High temperature (174 °C) resulted in high in-vitro digestibility (95%) but also high loss of EAA (EAA total 25%, Thr 43%, Lys 27% and Cys 93% respectively). Moisture had positive influence on EAA, Thr, Lys and Cys but no influence on protein digestibility. Residence time had positive effect on protein digestibility and negative on EAA.

Based on the obtained results a range of 120-140°C for temperature, 75% to 85% for moisture and 10-30 minutes for time resulted in optimal feather meal qualities respectively.

4.2.4 LOWERING PROTEASE INHIBITORS IN SPRAY DRIED EGGS IMPROVES PERFORMANCE OF WEANED PIGLETS

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With 47% protein and a favourable amino acid profile, spray dried eggs (SDE) are an interesting protein source for the feed industry. Several trials were done with SDE in piglets and calves but results were conflicting. Some trials showed a linear decrease in growth when the level of SDE increased whilst other trials did not see any effect. A suggested explanation is that eggs contain protease inhibitors like ovomucoid and ovoinhibitor which have binding sites for proteases as trypsin and chemotrypsin. When these proteases get bound to protease inhibitors, proteins in the gastro intestinal tract will not be broken down and thus cannot be used by the animal.

In an experiment the trypsin inhibition in SDE was reduced with 92%. This treated SDE (TSDE) was tested in weaned piglets against blood plasma (BP) (80% CP), whey protein concentrate (WPC) (35% CP) and SDE (47% CP). 480 Topigs 20/30 X Piétrain piglets were weaned at 27 days. Piglets were divided over the experimental groups based on weight, sex and ancestry. From weaning until 6 days after weaning the experimental diets were provided. Protein, fat and EW were kept at the same level. Daily weight gain in the first 6 days was significantly higher (P=0.01) in the BP group compared to the WPC and SDE group, TSDE was in the middle (respectively 197 vs. 144 vs. 151 vs. 172 g/piglet/day). The daily feed intake over this period was 225 g/piglet/day for BP, 196 g/piglet/day for TSDE, 188 g/piglet/day for SDE and 203 g/piglet/day for WPC. This resulted in better FCR's for the BP and TSDE groups (respectively 1.16 and 1.17) compared to the SDE and WPC group (respectively 1.42 and 1.38). It can be concluded that lowering protease inhibitors in SDE improves the technical results compared to SDE and WPC.

4.2.5 EFFECT OF GEL STRUCTURE ON THE *IN VITRO* GASTRIC DIGESTION OF PLANT PROTEINS

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Several studies indicate that digestibility of animal proteins is higher than plant proteins. However, it is also shown that plant proteins are nearly or equally as digestible as animal proteins. Along with the nature of the protein source, the food matrix can influence the protein digestibility. Here, we assessed both the effect of protein source as well as gel structure on *in vitro* gastric digestion. The gels were made from proteins of different plant and animal sources at various pre-treatment temperatures (90, 120 and 140 °C). We have used soy protein isolate (SPI), pea protein isolate (PPI), whey protein isolate (WPI) and albumin. *In vitro* gastric digestion was performed in simulated gastric juice at pH 2.0 and 37 °C. Samples of the gastric juice were taken at different times during 3 h and the degree of hydrolysis (OPA method) and peptide distribution (Size-exclusion chromatography) were measured. Also the hardness of the gel before and after digestion was analysed. Our results with dissolved proteins showed a higher degree of hydrolysis (DH) for SPI and PPI as compared to WPI and albumin. The results with protein gels showed a high digestibility of the PPI gel and a relatively low digestibility for the SPI and WPI gels. The hardness was significantly different for all studied gels. For both plant protein gels, SPI and PPI, highest temperature resulted in the weakest gel. We conclude that plant proteins present higher gastric digestibility than animal proteins. There is a relation between gel hardness and digestion rate.

4.2.6 *IN VIVO* AND *IN VITRO* STUDY ON THE MODE OF ACTION OF SPRAY DRIED PLASMA WHEN USED AS FEED ADDITIVE

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Spray dried plasma (SDP) is a protein-rich feed additive that is widely applied in pig production. Several studies have shown beneficial health effects of SDP. However, the molecular mechanism or processes underlying the beneficial effect of SDP is not fully understood. To better understand the mode of action by which SDP exerts its beneficial effects, six-week old pigs were fed for four-weeks with an experimental diet supplemented with porcine SDP. Genome-wide gene-expression profiles of ileal mucosa were compared to profiles generated from mucosa dissected from piglets fed with three reference diets supplemented with protein fractions from soybean, wheat gluten or rapeseed meal. In addition, gene-expression in cultured intestinal porcine epithelium cells (IPEC-J2) was measured after exposure to SDP for 2 and 6h. We observed a striking overlap in the SDP-induced responses between the *in vivo* and *in vitro* experiments. In both systems, SDP affected the remodeling and repair of the extracellular matrix in combination with the induction of a set of growth factors. In addition, SDP stimulated the innate (via Toll- and NOD-like receptors) and adaptive (via B-cell activity) immune system in combination with a set of cytokines. These results uncovered novel knowledge on the mode of action of SDP in its capacity to interact with the host and support the homeostasis of the host.

4.2.7 SUSTAINABLE PROTEIN PRODUCTION USING LESSER MEALWORMS

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Food production has been estimated to contribute with approximately 20-30% of the environmental impact of EU-citizens. In addition, the Food and Agriculture Organization of the United Nations (FAO) estimate that food production needs to increase 70% by 2050 to feed the growing global population, highlighting the importance of generating new and sustainable sources of high quality protein. FAO has recently placed food production from insects on the global agenda due to several advantages, e.g. high nutritional value (40-60% protein), high efficiency, low land and water requirements, and low climate impact. Thus, insect production is expected to be the next generation of sustainable livestock; yet, the production technology is still at sub-industry level.

Proti-Farm (formerly Kreca) is based in The Netherlands and is a leading international producer of various types of edible insects. Their flagship product is the lesser mealworm (LMW) which are sold, both whole and fractionized, for food and pharmaceutical applications. Presently, a large-scale production facility is under construction (with expected output of several thousand tons larval biomass per year); this is running consecutively with an EU based R&D project (SUSMEAL) together with Danish partners. SUSMEAL is investigating how to grow LMW in an automated manner suitable for cost-effective mass production, which is being accomplished by: i) developing low-cost nutritious LMW feed with consistently high protein and fatty acid content; and ii) integrating automation of production and monitoring of LMW health and maturity.

LMW nutrition is key to generate a protein-rich biomass with a high feed-conversion efficiency. Results on diet development for lesser mealworms based on using low-cost by-products in the feed composition will be discussed in the presentation, together with livestock management issues like disease control and housing conditions. Large scale insect rearing will be set in the global perspective of insects as next generation sustainable food production.

Session 5

Future challenges for sustainable protein supply and consumption

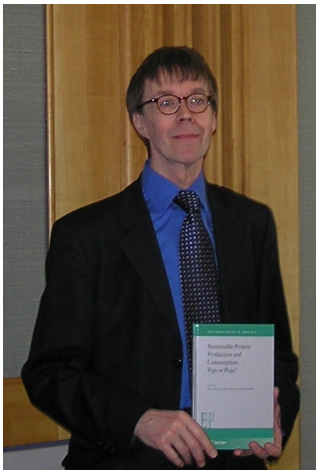
Wednesday, 26 October 2016

KEYNOTE LECTURE

5.1 SUSTAINABILITY AND PROTEIN PROVISION: DRAWING ON A DOZEN DIFFERENT DISCIPLINES

Harry Aiking, PhD, ERT Associate Professor, Chemistry & Food

Institute for Environmental Studies (IVM), VU University



In May 2014, Harry Aiking formally retired from VU University in Amsterdam as an Associate Professor in "Chemistry & Food". Continuing to supervise PhD students, he remains affiliated with the Institute for Environmental Studies (IVM-VU), where he has been leading dozens of multidisciplinary projects on the interface of natural and social sciences since 1980. From 1999-2005, he led the interdisciplinary NWO programme PROFETAS (Protein Foods, Environment, Technology And Society).

He published over 350 publications and has been a European Registered Toxicologist (ERT) since 1997. With a background in biochemistry and microbiology, he worked as a research associate at Indiana University in Bloomington (IN), USA. Subsequently, he became a KWF (Dutch Cancer Fund) Fellow at the Central Blood Bank Laboratory in Amsterdam before he joined IVM-VU.

In order to fully appreciate the intricate relationships between sustainability and protein, the input of at least a dozen different scientific disciplines is required, including political science, economics and psychology, as well as ecology, chemistry, nutrition science and medicine. This presentation starts by identifying ecological, economic and social aspects of food sustainability and food security, and by prioritizing the environmental impacts associated with food production and consumption. Subsequently, it is argued how nitrogen and protein are underlying and linking the top-3 environmental impacts, i.e. 1) biodiversity loss, 2) nitrogen cycle disruption, and 3) carbon cycle disruption (i.e. climate change). Addressing agriculture, food industry, consumers, and governmental stakeholders plus cultural aspects, future

challenges and options are sketched, with 2030 and 2050 as important waypoints. This keynote reflects these recent papers:

van Dooren, C., Douma, A., Aiking, H., Vellinga, P. (2017) Proposing a novel index reflecting both climate impact and nutritional impact of food products. *Ecological Economics* 131, 389-398.

de Boer, J., de Witt, A., Aiking, H. (2016). Help the climate, change your diet: A cross-sectional study on how to involve consumers in a transition to a low-carbon society. *Appetite* 98, 19-27.

van Dooren, C., Aiking, H. (2016). Defining a nutritionally healthy, environmentally friendly, and culturally acceptable Low Lands Diet. *International Journal of Life Cycle Assessment* 21, 688-700 (2016).

van Dooren, C., Tyszler, M., Kramer, G.F.H., Aiking, H. (2015). Combining low price, low climate impact and high nutritional value in one shopping basket through diet optimization by linear programming. *Sustainability* 7, 12837-12855.

Schösler, H., de Boer, J., Boersema, J.J., Aiking, H. (2015). Meat and masculinity among young Chinese, Turkish and Dutch adults in the Netherlands. *Appetite* 89, 152-159.

Aiking, H. (2014). Protein production: Planet, profit plus people? *American Journal of Clinical Nutrition* 100 (Suppl.), 483S-489S. ***[this review paper elucidates the foundations of this keynote]***

de Boer, J., Schösler, H., Aiking, H. (2014). "Meatless Mondays" or "less but better"? Exploring strategies to adapt Western meat consumption to health and sustainability challenges. *Appetite* 76, 120-128.

van Dooren, C., Marinussen, M., Blonk, H., Aiking, H., Vellinga, P. (2014). Exploring dietary guidelines based on ecological and nutritional values: A comparison of six dietary patterns. *Food Policy* 44, 36-46.

5.2 EXPLORING DIETARY GUIDELINES REFLECTING BOTH CLIMATE IMPACT AND NUTRITIONAL VALUES

Van Dooren, Corné

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'Existing official dietary guidelines almost all coincide with the dietary changes necessary to achieve an environmentally sustainable diet,' conclude the latest Nordic nutrition recommendations. Several European countries started to implement sustainability issues in their Food Based Dietary Guidelines. The Health Council of the Netherlands -for instance- came up with a general advice to 'Follow a dietary pattern that involves eating more plant-based and less animal-based food' and specific advices to add legumes and nuts to your diet. There is growing scientific evidence that diets rich in plant-based protein sources are nutrient rich and low in climate impact. It is important to develop new education models that ensures the food security and nutritional quality for the total population, within the existing planetary boundaries.

5.3 THE EFFECT OF EXERCISE ON INTESTINAL PERMEABILITY TOWARDS SMALL MOLECULES AND PROTEIN

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[5] Nutricia Research, Utrecht, The Netherlands

Strenuous exercise is associated with stress responses, such as impairment of intestinal integrity, possibly leading to increased permeability and altered absorption of dietary proteins. We examined to what extent cycling interventions result in intestinal permeability towards small molecules and proteins.

Twelve well-trained male volunteers (group A) ingested 40 grams of casein protein and a lactulose/rhamnose (L/R) solution in rest and after completing a two-step cycling protocol. This procedure was repeated one week later. In group B, ten healthy volunteers ingested 100 grams of peanuts and an L/R solution followed by rest or an endurance cycling protocol (group B). Intestinal permeability was measured as L/R ratio in 1h plasma. Excretion of casein-derived betacasomorphin-7 (BCM7) was measured in 5h urine. Ara h6, a major peanut allergen, was measured in serum. Several stress-related markers were measured before and after completing the procedure.

In group A, 1h plasma showed increased L/R ratio post-exercise during the first protocol execution, but not when the test was repeated. Likewise, stress-related markers were only significantly increased after the first protocol execution. In contrast, BCM7 levels in urine were higher post-exercise during both protocol executions. In group B, circulating levels of Ara h6 after peanut ingestion show remarkable differences between the resting and the exercise conditions.

We show that strenuous exercise leads to an initial increase in intestinal permeability and potential stress markers. This response seems to be adaptive already upon a single repetition. Independent of the adaptive stress response, an increased BCM7 accumulation in urine is seen, which prolongs and does not adapt. Endurance exercise also led to demonstrable uptake of Ara h6 (epitopes) after peanut consumption. These data indicate that different forms of strenuous exercise can increase appearance of nutritional protein and protein-derived peptides in the blood.

5.4 SUSTAINABLE PROTEIN SUPPLY FOR ANIMAL NUTRITION

Hendriks, Wouter

Wageningen University & Research, The Netherlands

5.5 CREATING SYNERGY: THE INDUSTRY OUTLOOK

Klein Essink, Gerard

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Poster presentations

PROCESSING INFLUENCE ON PROTEIN DIGESTION AND POST-ABSORPTIVE AMINO ACID UTILISATION IN GROWING PIGS

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During (over-)processing, reducing sugars can react with the free ε-amino group of lysine to form Maillard reaction products. Processing is known to reduce amino acid (AA) digestibility but it is unknown whether this is caused by a reduction in nitrogen solubility in chyme. Moreover, the post-absorptive effects of processing are unknown. Therefore, a series of experiments was conducted to determine the effects of processing on protein and AA digestion in the small intestine and post-absorptive utilisation of AA for retention in growing pigs. Soybean meal and rapeseed meal were used as protein sources. A model processing method, i.e. toasting at 95°C for 30 minutes in the presence of a sugar-rich lignosulfonate, was used to induce contrasts in protein quality. This was quantified by the reactive lysine content in the protein sources. The first study determined the standardized ileal digestibility (SID) of protein and AA of the protein sources using growing pigs fitted with an ileo-cecal valve cannula. Processing decreased the SID of protein and AA with the largest effect on SID of lysine and reactive lysine. The SID AA contents were used to formulate the diets of the second study. The second study determined the effects of processing on (1) nitrogen solubility in chyme, (2) apparent digestibility of protein along the small intestine, (3) body AA composition, and (4) post-absorptive AA utilisation for retention using a slaughter trial with growing pigs. Processing reduced apparent digestibility of protein especially at the end of the small intestine but this was not explained by effects on nitrogen solubility. Processing reduced AA retention and the lysine content in body protein. Processing reduced the post-absorptive AA utilisation, i.e. use of ingested SID AA for AA retention, for soybean meal but not for rapeseed meal. The interpretation and implications of these results will be addressed.

AMINO ACID DEMAND IN PIGLETS DURING POST-WEANING DIARRHOEA

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Current piglet diets are formulated according to the recommendations of the NRC. The recommended levels however might not be sufficient in case of gut villi atrophy and activation of the immune system that occur after weaning especially in case of post-weaning diarrhoea due to enterotoxigenic E. coli (ETEC). The current study tested the hypothesis that pigs challenged with ETEC will improve performance by dietary supplementation of amino acids above the current recommended levels. A total of 60 male weaned piglets (tested for susceptibility towards ETEC O149:F4ac binding in the gut) were stratified into one of 4 treatments. Four diets were formulated with either elevated levels of amino acids with focus on the support of the immune system (IMMUN), support of the gut (GUT), or a combination of the two (GUT IMMUN) and compared to a control diet based on the recommendations of the NRC (CON). Piglets were individually housed and were fed diets for 21 days after weaning. On days 5, 6, and 7 after weaning piglets were infected with ETEC (serotype O149:K91: K88). Piglets fed the IMMUN diet showed an improved growth, less diarrhoea and better health score after the ETEC challenge. Also urea levels in the blood were lower for the IMMUN group in this period, while haptoglobulin levels in the blood were significantly lower for this group in the week prior challenge.

It can be concluded that piglets with dietary supplementation of amino acids that support the immune system show a lower level of an indicator of inflammation immediate post-weaning and a better performance after an infected with ETEC.

A HYBRID DRY AND AQUEOUS FRACTIONATION METHOD TO OBTAIN

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Combination of dry and aqueous fractionation is investigated to obtain protein-rich fractions from quinoa in a milder and more sustainable way compared to conventional wet fractionation. Dry fractionation of quinoa involved milling and subsequent air classification, generating a protein-enriched embryo fraction. Subsequently, this fraction was milled, suspended, and further fractionated by aqueous phase separation.

The efficiency of aqueous phase separation could be improved by addition of NaCl (0.5 M). Finally, the top aqueous phase was decanted and ultrafiltered, resulting in a protein purity of 59.4 w/dw% for the 0.5 M NaCl-protein solution and a protein yield (gram protein obtained/gram protein in seed) of 62.0 %. Having used 98 % less water compared to conventional wet extraction, the hybrid dry and aqueous fractionation is a promising method for industry to create value from quinoa in a more economic and sustainable friendly way while minimizing the impact on quinoa's native protein functionality.

INTRINSIC ^{15}N LABELLING OF BOVINE MILK PROTEIN FOR THE DEVELOPMENT OF A DUAL STABLE ISOTOPE-BASED METHOD TO MEASURE DIETARY PROTEIN QUALITY

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The determination of protein quality has been identified as a critical question by international authorities (FAO, 2014). The current method for determining protein quality, measurement of the digestible indispensable amino acid score, is difficult because of the inaccessibility of the ileum. Therefore, we aim to develop a meal/plasma signature dual stable isotope-based approach for evaluating protein quality. For this purpose, we produced ^{15}N-labelled milk protein. Two Holstein Friesian dairy cows were infused with ^{15}N-ammonium sulphate (98%, 350g per cow) via a rumen cannula for 114 hours. To increase the efficiency of conversion of label into milk protein, cows were allowed ad libitum access to diets limiting in intestinal digestible protein. The contents of intestinal digestible protein and rumen-degraded protein balance were 82 and -12g/kg dry matter, respectively. Milk was collected during the entire study period. All raw milk collected after 48 hours of infusion was pooled for the production of milk protein concentrate. Total enrichment was determined by isotope-ratio mass spectrometry. After the start of ^{15}N infusion, the milk enrichment increased and steady state was reached after approximately 72 hours. Once this state was reached, efficiency of tracer incorporation in milk proteins was 20%. In raw milk, a peak ^{15}N enrichment of 2.31(±0.17) atom-% excess(APE) was detected at the end of the infusion period. The produced milk protein concentrate of this study showed an enrichment level of 1.90APE. These results show that rumen infusion with a highly enriched nitrogen source, together with dietary adjustment, leads to highly enriched ^{15}N-labelled milk compared to enrichment levels reported in literature. The milk protein concentrate will be used in a human study, the ^{15}N-labelled milk protein will be ingested together with ^{13}C-labelled Spirulina algae to test the dual stable isotope-based approach.*

*First results of this human study will likely be available at the conference.

Author index

Author index

L

M

N

O

P

R

S

T

V

W

Z